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IL-3 Receptor Alpha Chain is a Biomarker and a Therapeutic Target of Myeloid Neoplasms

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Abstract

The alpha-chain of the interleukin-3 receptor (IL-3RA or CD123) is frequently deregulated in myeloid neoplasias and its deregulation contributes to survival and proliferation of malignant cells. This review is focused to the analysis of the diseases in which CD123 is markedly overexpressed, such as blastic plasmocytoid dendritic neoplasms (BPDCN), systemic mastocytosis (SM), chronic myeloid leukemia (CML) and acute myeloid leukemia (AML). The highly overexpressed IL-3R represents also a molecular target suitable for the development of specific targeted therapies using either the IL-3 ligand fused with cytotoxic drugs, or mono-, bi- or tri-specific anti-CD123 monoclonal antibodies or CD123-specific chimeric antigen receptor T cells.

Keywords: Blastic plasmocytoid dendritic neoplasms; Systemic mastocytosis; Chronic myeloid leukemia; Acute myeloid leukemia; Chimeric antigens

Introduction

The Interleukin-3 (IL-3) Receptor (IL-3R), together with IL-5 and Granulocyte-Macrophage Colony Stimulating Factor Receptor (GM-CSFR), form a subfamily of cytokine membrane receptors, called the Beta Common (β_c) family of cytokine receptors, due to the presence of a common signaling β_c. The IL-3R is a heterodimer composed by an alpha chain, also known as CD123, and a common β_c. CD123 is expressed at high levels only on plasmocytoid dendritic cells and basophils, but at lower levels on monocytes, eosinophils and myeloid dendritic cells. CD123 expression was explored in detail at the level of the stem/progenitor cell compartment, showing that high CD123 expression characterizes myeloid and B-lymphoid progenitors, while low levels are expressed on erythroid and multipotent progenitors [1-4]. Common lymphoid progenitors and NK lymphoid progenitors are CD123-negative [5].

A part of $CD34^+CD38^-$ cells isolated from cord blood or bone marrow expresses CD123, its expression being higher on cord blood than on bone marrow cells [6]. Other studies have confirmed the low expression of CD123 on $CD34^+CD38^-$ cells isolated from either cord blood or bone marrow [7]. *Ex vivo* treatment of $CD34^+$ bone marrow cells with a neutralizing anti-CD123 mAb elicited a reduction of about 30% of the number of HSCs repopulating NOD/SCID mice [8].

The binding of IL-3 to the IL-3R induces a stimulation of cell survival, proliferation and differentiation.

CD123 expression is deregulated in many hematological malignancies. Particularly, CD123 was found to be markedly overexpressed in several hematological malignancies, including acute myeloid leukemia (AML), Hairy Cell Leukemia (HCL), Blastic Dendritic Cell Neoplasm (BCDN) and Chronic Myeloid Leukemia (CML) [reviewed in [9]. These observations have suggested that CD123 could represent a potential target in the therapy of these pathological conditions. The analysis of the experimental studies performed in these four malignancies represents the object of the present review.

CD123 in blastic plasmocytoid dendritic cell neoplasm

BPCDN is an aggressive neoplastic disease due to the malignant proliferation of plasmocytoid dendritic cells. BPDCN is a rare subtype of leukemia/lymphoma, classified in 2008 by the World Health Organization as a distinct pathologic entity in the context of AMLs and related precursors neoplasms. At anatomo-pathological level, the majority of BPDCN patients exhibits cutaneous lesions characterized by the presence of numerous immature blastic cells displaying typical features of plasmocytoid dendritic cells. At immunophenotypic level, the tumor cells are characterized by CD4 and CD56 positivity, strong CD123 positivity, CD304, TCL1 and CD303 positivity [10]. In addition to this mature blast phenotype, in some patients an immature plasmocytoid dendritic cell phenotype was observed, usually with tumor cells $CD56^-$ and typically in the absence of extramedullary disease at presentation. This heterogeneity of the maturational profile of BPDCN is reflected and translated into a considerable variability of the clinical spectrum, ranging from acute leukemia to mature lymphoma-like presentation [11].

As above mentioned one of the typical features of BPDCN consists in the particularly high CD123 expression: Granache-Ottou provided clear evidence that CD123 expression on BPDCN cells (both with typical and atypical phenotypic features) was markedly higher than that reported in other hematological neoplasia known to overexpress this membrane receptor [12]. It is important to note that 100% BPDCN cells strongly overexpress CD123 on their membrane, with mean expression levels being 6-10 times higher than those observed on AML blasts [12]. Furthermore, BPDCN cell survival and proliferation is stimulated by IL-3 [12].

These observations provided a strong rationale for evaluating first in pre-clinical and then in clinical settings the sensitivity of BPDCN cells to CD1213-targeting agents. In preclinical studies the *in vitro* and *in vivo* sensitivity of BPDCN cells to SL-401, a biological agent comprised of human IL-3 coupled to a truncated diphtheria toxin payload, was explored, showing that: BPDCN cells are exquisitely sensitive to the *in vitro* SL-402-mediated cell killing, their sensitivity

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being higher than that displayed by AML blasts; treatment with SL-401 markedly increases the survival of NOD-SCID mice injected with BPDCN cells [13]. These observations strongly supported the clinical use of SL-401 in BPDCN patients. SL-401 was evaluated in a phase I study in patients with advanced BPDCNs: the treatment with a single cycle of administration of SL-401 was well tolerated by patients and elicited a pronounced anti-tumor response, with 5/9 patients achieving complete responses and 2/9 partial responses [14]. On November 2015, the European Medicinal Agency has granted orphan drug designation to SL-401 for the treatment of BPDCN.

CD123 in systemic mastocytosis

Systemic mastocyotisis (SM) is a clinical condition characterized by the uncontrolled proliferation and accumulation of neoplastic mast cells at the level of various tissues, particularly bone marrow, skin and visceral organs [15]. The extent of organ infiltration and the consequent organ damage determine at large extent the subdivision and classification of the disease in 5 different subgroups: indolent systemic mastocytosis (ISM), smoldering SM (SSM), SM with associated clonal hematologic non-MC lineage disease (SM-AHNMD), aggressive SM (ASM) and MC leukemia (MCL) [15]. More than 90% of patients with SM possess mutations of the KIT receptor, involving a gain-of-function of the tyrosine kinase activity, due to an aspartic acid to valine substitution (D1816V), at the level of the second catalytic domain: this mutation determines a constitutive activation of the mutated KIT receptor and consequently a stimulation of survival and proliferation of transformed mast cells [reviewed in 16]. Recent next generation sequencing studies have revealed the presence of several additional genetic and epigenetic alterations in a subset of SM patients with advanced disease; particularly, these mutations involve signaling molecules (JAK2, KRAS, NRAS), epigenetic regulators (TET2, EZH2, DNMT3A, ASXL1), transcription factors (RUNX1) or splicing factors (U2AF1, SRSF2, SF381 [16]. Molecular profiling of the mutations present in myeloid progenitors of various types of SM patients provided evidence supporting in ASM and SM-AHNMD patients the occurrence of TET2, SRSF2 and ASXL1 mutations before KITD816V mutations [17].

CD123 could represent a potentially important target of leukemic cells in SM disorders. CD123 is not expressed on normal or reactive mast cells [18]. However, $CD34^+CD38^-$ cells isolated from SM patients clearly display expression of CD123 [19]. Initial studies based on the detection of CD123 expression by flow cytometry and immunohistochemistry have provided conflicting evidence about the expression of CD123 in SM disorders [20,21]. However, two recent studies showed that CD123 is clearly overexpressed on neoplastic mast cells. In an initial preliminary report, Pardanani and coworkers analyzed 6 SM cases showing that 5/6 displayed a high reactivity of neoplastic mast cells with anti-CD123 mAb, with 82-91% of positive cells; importantly, the mean fluorescence intensity of CD123 labeling on neoplastic mast cells was high, thus indicating a high level of expression [22]. More recently, the same authors have provided a more detailed analysis of CD123 immunostaining patterns in various types of SM showing that CD123 expression was constantly observed in patients with ASM, and in the majority of patients with ISM (61%) and SM-AHNMD (57%), while it was not expressed in patients with MCL [23]. Furthermore, two observations suggest that the level of CD123 expression on neoplastic mast cells may have an impact on disease progression and prognosis: in fact, focal proliferation of plasmocytoid dendritic cells around mast cell aggregates was frequently observed and with a higher frequency among CD123-positive than CD123-negative patients [23]; furthermore, the percentage of positive cells and the intensity of labeling had prognostic value both in ASM and SM-AHNMD patients [23]. These observations are clinically relevant, given the lack of efficacious treatments for patients with ASM or advanced disease and suggest that SM may offer a unique opportunity for CD123-targeting treatment, as indicated by the aberrant expression of this membrane receptor on both bulk neoplastic mast cells and $CD34^+CD38^-$ leukemic stem/progenitor cells.

CD123 in chronic myeloid leukemia (CML)

CML is a malignant chronic neoplasia caused by the formation of a driving oncogene, known as the fusion gene BCR-ABL1, originated by the exchange of genetic material due to the translocation between chromosomes 9 and 22. BCR-ABL1 was sufficient to induce leukemia formation and to induce the uncontrolled proliferation of hematopoietic stem cells. The discovery of this protein has led to the development of tyrosine kinase specific inhibitors (TKIs), whose administration to the patients during the chronic phase was able to manage CML development. However, this treatment was not curative since quiescent, dormant CML leukemic stem cells (LSCs) are resistant to the treatment with TKIs [24]. Various strategies have been proposed to try to kill dormant CML stem cells. A regulatory pathway involving peroxisome proliferator-activated receptor-γ (PPARγ), the transcription factors STAT5 and HIF-2α and the protein CITED2 (a transcription factor involved in stem cell maintenance), maintains the survival of CML stem cells [25]. Thus, it was shown that CML LSC pool can be purged by glitazones, antidiabetic drugs acting as agonists of PPARγ: following PPARγ activation, expression of STAT5 and of its direct targets HIF-2α and CITED2 is decreased [25].

Recent studies indicate that CD123 overexpression is a distinctive feature of CML stem cells compared to normal HSCs. In fact, Hermann and coworkers reported that CML $CD34^+CD38^-$ cells express high levels of CD123 [26]. Given these observations, preclinical studies have evaluated the impact of CD123-targeting agents on CML LSCs. Thus, Frolova and coworkers reported the killing of $CD34^+CD38^-$/$CD123^+$ CML progenitor cells from primary CML cells, including those harboring ABL T315I mutation [27], with SL-401 or SL-501. Interestingly, these IL-3R targeting agents were able to decrease the number of primitive long-term culture-initiating cells in samples of advanced CML, TKI-resistant [27]. In another report, Nievergall and coworkers reported that CD123 expression is elevated in CML progenitors and stem cells, compared with healthy donors; interestingly, CD123 expression in $CD34^+CD38^-$ cells was higher in blastic crisis than in chronic phase CML patients [28]. Monoclonal antibody targeting of CD123 with CSL362 antibody resulted in an efficient depletion of CML progenitors and stem cells [28].

These observations support the experimental use of IL-3R targeting drugs as agents attempting to eradicate the leukemic stem cell population in CML.

CD123 in acute myeloid leukemias

CD123 is frequently overexpressed in AMLs and represents a marker for leukemic stem cells. This topic was recently reviewed and analyzed in detail [29,30]. Thus, here we will limit our analysis to the currently used approaches to target leukemic $CD123^+$ cells.

CD123 was clearly overexpressed in about 50% of the AMLs at the level of the bulk blast cell population [31]; CD123 is clearly expressed on $CD34^+CD38^-$ leukemic cells, while the normal $CD34^+CD38^-$ cell population scarcely expresses this membrane antigen [8,32]; CD123 expression on AML blasts confers a survival/growth advantage to leukemic cells and is related to a negative outcome [31,33].

IL-3R targeting using IL-3

One approach to target IL-3R present on the surface of leukemic blasts consists in the use of the IL-3, the natural IL-3R ligand, coupled with an anti-leukemic drug or with any other molecule capable of exerting a cytotoxic effect. One of these compounds is DT_{388}IL-3, a recombinant toxin obtained through the fusion of the catalytic and translocation domains of Diphteria Toxin (corresponding to the amino acid residues 1-388, DT_{388}) through a Met-His linker, to human IL-3 [34]. A variant of this fusion toxin was obtained, resulting in the new fusion toxin DT_{388}IL-3[K116W], with enhanced binding affinity for IL-3R [35]. Both DT_{388}IL-3 and DT_{388}IL-3[K116W] are able to induce both *in vitro* and *in vivo* a pronounced cytotoxic effect against IL-3R-positive leukemic cells, the level of cytotoxicity being clearly correlated with the level of receptor expressed on the surface of leukemic cells (both α and β chains) [36,37]. DT_{388}IL-3 was well tolerated *in vivo* up to 100 μg/kg [38].

Based on this promising pre-clinical background, the IL-3 cytokine-toxin fusion protein was developed as a drug (SL-401) and tested in phase I clinical trials. Some clinical studies were specifically designed to evaluate the safety profile and the potential therapeutic impact of SL-401 in a group of heavily pretreated AML patients [39]. SL-401 was administered to a total of 70 AML patients, showing two complete responses and five partial responses and an improved overall survival, compared to historical controls [39]. In some treated AML patients a durable, stable disease condition was observed for more than 1 year [39]. An ongoing expansion study is planed in AML patients, using SL-401 at 12 μg/kg/day [40].

CD123 targeting with neutralizing mAbs

A second strategy consisted in the development of mAbs able to interact with high-affinity and to block IL-3R. In this context, Sun et al. reported the development of a mAb, 7G3, able to interact with IL-3R and to block IL-3-mediated activation of this receptor [8]. Through the targeting of CD123, this antibody impaired the growth *in vivo* of $CD123^+$ leukemic cells, including LSCs, while it affected only marginally HSCs [8]. The CSL362 antibody was developed from the anti-CD123 mAb 7G3 in a process of progressive "humanization", affinity optimization and Fc engineering to improve affinity of binding to the FcR CD16 [41]. This antibody mediates antibody-dependent cell-mediated cytotoxicity (ADCC) of AML blasts, including leukemic stem cells, sparing normal pluripotent progenitors/stem cells [42]. CSL362 synergizes with anti-leukemic drugs to inhibit the growth of primary AML cells grafted into NOD-SCID mice [43].

A phase I study evaluated the safety, pharmacokinetics and preliminarily also the anti-leukemic activity of CSL360 in 40 relapsed, refractory AML patients, showing that only 2 patients responded to this treatment: this observation suggested that in high-risk AML patients IL-3R blockade induced by this antibody, when used alone, was insufficient as a therapeutic strategy [44]. A second phase I study using CSL362 mAb was carried out in AML patients who have achieved a complete remission after standard treatment, but are at high risk of relapse. In the first 25 treated patients at a follow-up at 6 months: 50% of the patients maintained a CR; interestingly, 3 of 6 patients with detectable MRD at baseline converted to negative on study [45]. 12 of these patients displayed Minimal Residual Disease (MRD^+); 4 of these patients converted to a MRD^- condition following treatment and remained in CR at week 24, while the 7 patients who did not convert to a MRD^- condition following treatment relapsed prior to wk 24 [45]. This MRD conversion suggests a possible eradication of residual leukemia cells [45]. Multiple factors may influence the response to therapy with CSL362: major determinants of this response are related to the expression of the target receptor on LSCs, MRD leukemic cells and the number and immunological activity of NK lymphocytes [45].

Dual-affinity re-targeting (DART) proteins are a class of bi-specific mAbs consisting of two peptides each composed of the variable heavy chain region of one antigen recognition site linked to the variable light chain region of a second antigen recognition site; the resultant heterodimeric protein is stabilized by a C-terminal disulfide bond between the two antibody chains. Using this technology, it was reported the development of bi-functional fusion anti-CD123 and anti-CD3 antibodies (CD123 × CD3 bispecific scFv). These fusion antibodies displayed several interesting biological properties suitable for clinical applications, including high target cell-binding affinity, increased *in vivo* stability and capacity to drive T-cell mediated target cell killing [46]. Recent pre-clinical studies reported the development of a bi-specific CD3 × CD123 DART molecule (MGD006), able to bind both to T lymphocytes and to cells expressing CD123 [47]. MGD006 was shown to be able to induce *in vitro* depletion of primary AML blasts, concomitantly with activation of residual T cells and *in vivo* anti-leukemic activity in leukemia-bearing mice; its administration was well tolerated in primate models (cynomolgus macaques), inducing a transient cytokine release after the first infusion and no significant changes in blood cell counts, with the exception of a transient decrease in RBC counts [47]. In another recent study, the effect of MGD006 on primary AML blasts using an *in vitro* methodology based on the culture of leukemic blasts on stromal layers was investigated: treatment with MGD006 was able to induce a marked decrease of total leukemic blasts cells, as well as $CD34^+$ or $CD123^+$ cells [48]. Furthermore, incubation of normal cord blood $CD34^+$ cells for 18-24h with MGD006 did not alter their capacity to act as HPCs, i.e., to generate hematopoietic colonies [48].

An ongoing phase I clinical trial is evaluating the safety, tolerability and anti-leukemic activity of MGD006 in patients with relapsed or refractory AML.

In addition to CD3xCD123 bispecific antibodies, other studies have attempted to the development of either bispecific CD123 × CD16 antibodies, or tri-specific CD123 × CD33 × CD16 antibodies. Thus, a first study reported the development of a conjugate of single-chain Fv antibody fragments specific for CD123 and CD16: through its anti-CD16 moiety this antibody binds to NK lymphocytes and monocytes and drives these cells on $CD123^+$ targets, such as leukemic cells, inducing their killing [49]. The same group of investigators reported the development of a tri-specific antibody composed of a single-chain Fv derivative with anti-CD123, anti-CD16 and anti-CD33 specificities: this tri-specific antibody is able to induce the killing of $CD123^+$ and $CD33^+$ AML cells, including LSCs [50]. The peculiar properties of this antibody are particularly interesting for their potential potent targeting of some AML subtypes, such as $FLT3\text{-}ITD^+$-AMLs and NPM1-mutated AMLs [51,52]. It is important to note that these AMLs are characterized by a pronounced CD123 overexpression [51,52].

T-Cells expressing CD123 Chimeric antigen receptors (CARs)

Adoptive cell transfer of T cells genetically engineered with tumor-reactive antigen receptors (CARs) is a rapidly developing strategy in experimental oncology. CARs are monomeric receptors build-up by fusing the single-chain fragment variable (scFv) of a tumor-reactive antibody with a transmembrane domain and one or more signaling molecules containing intracellular immunoreceptor tyrosine-based activation motifs (ITAMs). As a consequence of CAR modification, T technology is related to the potency of T-cell mediated effect on tumor

antigen-bearing cells, to their bio-distribution *in vivo* and long-term persistence [53].

The CAR technology developed rapidly and three generations of CARs according to their structure can be distinguished: the first generation consisted of an antibody-based external receptor structure and a cytosolic domain including the ITAM from TCRζ or FcRγ; the second generation included co-stimulatory signaling domains, such as CD28; third generation includes three or more cytosolic domains with multiple co-stimulatory domains [54]. This technology has found a very interesting application in the treatment of patients with relapsed or refractory B cell malignancies treated with CD19-specific CART cells, achieving in some treated patients durable remissions [54]. These clinical studies have also provided indication that, while the permanent genetic modification of T lymphocytes seems to be safe, the modulation and management of their function *in vivo* is complex and difficult, mainly due to the induction of secondary effects related to activation of the immune system [54]. Recent experimental studies have proposed some strategies for controlling *in vivo* the activity of engineered T cells [55].

Given the success obtained in ALL using CD19-specific CART cells, it is highly desirable to find a CAR target to treat AMLs. A potential candidate is CD123. Thus, various attempts have been made to develop CD123-specific CART cells and to test their effect on AML cells and on the normal stem/progenitor cell counterpart. Using this approach, Tettamanti et al reported in a first study the development of cytokine-induced killer (CIK) cells by transducing T cells with a retroviral vector encoding an anti-CD123 chimeric receptor and observed that transduced cells were able to exert a cytotoxic activity against AML cells, including leukemic progenitor/stem cell populations [56]. In a second more recent study the same authors reported the development of both anti-CD123 and anti-CD33 CAR T cells and evaluated the immunotherapeutic potential *in vivo* in the NOD/SCID IL2Rγ$^{-/-}$ mouse model xenografted with primary human AML cells: anti-CD123 CAR T cells were superior to anti-CD33 CAR T cells to induce *in vivo* clearing of AML cells and have a more favorable toxicity profile, inducing a limited toxicity against normal HSCs of either cord blood or adult bone marrow [57]. Since CAR T cells targeting CD33 resulted in an unacceptable cytotoxicity in xenograft models of hematopoietic toxicity, it was designed a transiently expressed mRNA anti-CD33 CAR, resulting in the generation of CD33-specific CAR T displaying potent, but self-limited activity against AMLs [58].

In another study the development of two CARs containing a CD123 specific scFv in combination with a CD28 co-stimulatory domain and CD3-related signaling domain was reported: CD123-redirected T cells with this vector displayed potent cytotoxic activity *in vitro* and *in vivo* against CD123^{+} AML cells [59]. Importantly, T lymphocytes obtained from AML patients were manipulated to express CD123 CARs and were shown to induce the killing of autologous AML blast cells [59].

In a last study it was reported the development of CD123-specific CAR T using scFv sequences from two different antibodies linked to the 4-IBB and CD3ζ chain signaling domain [60]. CAR T cells thus obtained when incubated in the presence of primary AML cells undergo activation, with consequent release of cytokines and killing of the leukemic cells [60]. A very remarkable property of these CD123-specific CAR T cells consisted in their capacity to induce the efficient killing also of cells with dim CD123 expression and, due to this property, to eradicate *in vivo* leukemic growth in xenograft leukemia mouse models [60]. Importantly, this study investigated also the effect of CAR T 123 immunotherapy on NSG mice reconstituted with normal human fetal liver CD34^{+} cells, resulting in eradication of normal myelopoiesis [60]. This last finding raises two important problems, one related to the potential use of CAR T 123 as a novel valuable conditioning regimen prior to hematopoietic stem cell transplantation, and the other related to the concerns relative to the clinical use of these CAR T 123 cells, requiring a rescue strategy [60]. Concerning the last point it is important to point out that two different studies have shown opposing effects of anti-CD123 CAR T on normal human CD34^{+} cells [57,60]. However, as pointed out by Tettamanti and coworkers [61] a remarkable difference between these two different studies was related to the different source of normal human CD34^{+} cells used in these studies; in fact, Pizzitola and coworkers used human cord blood and adult bone marrow CD34^{+} cells, while Gill et al used human fetal liver CD34^{+} cells [61]. It would be possible that fetal liver CD34^{+} cells express CD123 at higher levels than normal cord blood or bone marrow CD34^{+} cells [61].

Given these concerns about potential toxicities of CD123 T CARs for normal CD34^{+} cells, the translation of anti-CD123 CAR to the clinic will require the development of switch-off mechanisms, such as suicide mechanism, to eliminate effector T cells after that these cells have mediated their anti-tumor effects.

Conclusion

The studies carried out in the last years have provided evidence that CD123 is overexpressed in some myeloid neoplasms, including BPDCN, CML, SM and AML. The high expression of CD123 in these conditions is an important biomarker and offers also an important opportunity for targeting tumor cells. Thus, various agents targeting CD123 are under development and SL-401 (IL-3 ligand fused with diphtheria toxin) is already a valuable drug for the treatment of relapsing BPDCN patients. Other IL-3R-targeing drugs, such as neutralizing CD123 mAbs, have shown preliminary evidence of efficacy for the treatment of MRD in AML patients.

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